

# Retinal vascular changes following supplementation with alpha-tocopherol or beta-carotene

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## ABSTRACT.

**Purpose:** To examine if long-term supplementation with alpha-tocopherol (AT) or beta-carotene (BC) was associated with the prevalence of vascular changes in retinal arterioles.

**Methods:** An end-of-trial subsample from a double-blind, placebo-controlled clinical trial designed to study the effects of alpha-tocopherol and beta-carotene on lung cancer incidence (ATBC Study).

**Setting:** Source population of Helsinki and the surrounding province.

**Participants:** 1072 men 50-69 years old and smoking at least 5 cigarettes per day at study entry.

**Interventions:** Random allocation to one of four supplementation regimens: 50 mg per day alpha-tocopherol, 20 mg per day beta-carotene, both alpha-tocopherol and beta-carotene, or placebo. Median follow-up time was 6.6 years (range 5.2-8.0 years).

**Main outcome measure:** Presence of vascular changes in retinal arterioles as determined from end-of-trial retinal color photographs.

**Results:** Retinal vascular changes were most prevalent in the AT (161 men, 62%), and in the BC (163 men, 62%) groups. The prevalence rate was lowest in the AT plus BC group (161 men, 55%), and slightly higher in the placebo group (145 men, 57%). There was no statistically significant association of either AT (OR 0.9, 95% CI 0.7-1.2) or BC (OR 1.0, 95% CI 0.8-1.3) supplementation with the prevalence of retinal vascular changes after adjusting for major risk factors.

**Conclusions:** Supplementation with alpha-tocopherol or beta-carotene for a median of 6.6 years does not protect against retinal vascular changes among smoking males.

**Key words:** alpha-tocopherol – beta-carotene – antioxidant – retina – vascular – supplementation.

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Hypertensive or arteriosclerotic changes in retinal arterioles both involve thickening of the vessel walls caused by medial layer hypertrophy, hyalinization in the intima, and hyperplasia in the endothelial layer. Similar arteriolar changes are detectable at the same time

in the brain, heart, and kidney. Changes caused by arterial hypertension or arteriosclerosis cannot be separated by the clinical appearance of the retinal arteries. We refer to “retinal vascular changes” in this study without any emphasis as to their origin.

Primarily, the changes of hypertension are due to vasospasm, while those of sclerosis result from thickening of the retinal vessel wall. Increased thickening of the retinal arteriolar wall in conjunction with a sclerotic process causes progressive changes in the appearance of the retinal arterioles. Vascular sclerosis and perivascular glial proliferation lead to the typical “arteriovenous nicking” (Klein et al. 1994). In arterial hypertension the high perfusion pressure increases blood flow in retinal arterioles and narrowing is observed (Garner et al. 1975, Hayreh et al. 1986).

Changes in retinal arterioles have been associated with left ventricular thickening (Dahlöf et al. 1992), atherosclerosis (Salus 1958), systemic hypertension (Klein et al. 1994), internal carotid artery occlusion (Hollenhorst 1958, Kearns & Hollenhorst 1963), age (Klein et al. 1994), and increased mortality (Svardsudd et al. 1978).

Free radical formation and oxidation are believed to be associated with many age-related degenerative diseases. Free radical production is increased by smoking, inflammation, and UV-radiation (Fridovich 1984). A key event in both the initiation and progression of arteriosclerosis seems to be oxidation of LDL (Witztum & Steinberg 1991, Steinberg & Witztum 1990). Antioxidants are assumed to protect LDL against oxidation (Steinberg et al. 1989).

Animal studies have shown a decrease in atheroma formation in animals fed with vitamin E compared with controls (Smith et al. 1989, Wojcicki et al. 1991, Verlangieri & Bush 1992). In uncon-

trolled studies the use of vitamins or micronutrients with antioxidant potential has been associated with a decreased risk of coronary artery disease (Rimm et al. 1993, Stampfer et al. 1993). Correspondingly, low plasma levels of ascorbate, tocopherols, and beta-carotene have been associated with elevated risk levels of cardiovascular disease (Gey et al. 1991, Manson et al. 1991, Riemersma et al. 1991).

Direct observation of retinal arterioles provides a non-invasive method of evaluating arteriosclerosis and the effects of systemic hypertension. We report the results of a clinical analysis of retinal vascular changes in an end-of-trial random subsample of a controlled, long-term clinical trial of two powerful antioxidants, alpha-tocopherol (AT) and beta-carotene (BC).

## Material and methods

### Parent ATBC Study

Recruitment into the ATBC Study took place between 1985 and 1988. The participants were enrolled from the total male population aged 50 to 69 years in southwestern Finland ( $n=290406$ ). They had to be current smokers of at least 5 cigarettes per day at entry. Exclusion criteria were: history of cancer or other serious disease limiting participation, use of vitamin E, vitamin A, or beta-carotene supplements in excess of defined doses, and anticoagulant treatment. Accordingly, 29,133 men were randomized to one of the four supplementation regimens: alpha-tocopherol (50 mg/day,  $n=7278$ ), beta-carotene (20 mg/day,  $n=7282$ ), both alpha-tocopherol and beta-carotene ( $n=7278$ ), or placebo ( $n=7287$ ). This two-by-two factorial design allowed the effects of the two supplements to be assessed independently in one study. The study agents were formulated as synthetic dl-alpha-tocopheryl acetate (50 per cent powder) and synthetic beta-carotene (10 per cent water-soluble beadlets) and the daily dose was given in a single capsule. Intervention continued until April 30, 1993. A detailed description of the study rationale, design and methods, as well as eligibility, randomization and characteristics of the participants, and measures of compliance, has been published previously (The ATBC Cancer Prevention Study Group, 1994a).

At baseline, data on background characteristics, medical and smoking his-

stories, and diet were obtained by a questionnaire. In addition height, weight and blood pressure were measured, and a blood sample was drawn and the serum was stored deep-frozen at  $-70^{\circ}\text{C}$ . Visual acuity was measured using the Snellen visual acuity charts, with and without any glasses for far vision. Alcohol consumption and dietary intake of vitamin E and beta-carotene were estimated from a diet history questionnaire (Pietinen et al. 1988). Serum cholesterol was determined enzymatically (CHOD-PAP-method, Boehringer, Mannheim). High-performance liquid chromatography was used to measure the serum levels of alpha-tocopherol and beta carotene (Milne et al. 1986).

The follow-up consisted of three visits per year to a local study center. The measurement of visual acuity was repeated at every third visit, i.e. once a year. At each visit the participants returned the capsule pack from the previous period and were given a new one. Compliance was assessed by counts of the remaining capsules at each visit. The overall compliance was good, with four out of five active participants taking more than 95 per cent of their capsules. There were no differences in capsule consumption across the intervention groups. The proportion of participants leaving the study for any reason (31%), including death, did not differ between the supplementation groups.

### Study sample

The subjects for this study were drawn from the participants of the Helsinki and surrounding Uusimaa areas attending their last ATBC Study follow-up visit between November 1992 – March 1993. All men over 65 years at that point were included. In addition, a random sample of 129 participants younger than 65 years was added, totalling 1072 men. A clinical ophthalmological examination was performed on these men. A pre-examination questionnaire assessed prior ocular history. Written informed consent was obtained from every participant separately for this ophthalmological study.

### End point assesment

The clinical ophthalmological examination involved dilatation of the pupils and color fundus photography. Three pictures were taken from each eye using magnifications of 40 (two pictures) and

60 (one picture) degrees. Any vascular changes in retinal arterioles were assessed from these photographs. A retinal specialist in our group (LL) performed the evaluation. The classification was as follows: 0) normal, 1) mild changes in form of broadening of the central light reflex with or without arteriovenous nicking, and 2) moderate to severe changes in the form of severe arteriolar narrowing and moderate to severe arteriovenous nicking. A person was classified as having retinal vascular changes if he had changes in either eye.

Participants and all study staff remained masked to the participant's supplementation throughout the trial. Similarly, the examiner of the color fundus photographs was given no information on the participant other than the photographs.

### Risk factors

Age was defined as age at the time of the clinical ophthalmological study. History of diabetes, claudication and coronary heart disease, use of alcohol, cigarette smoking, body mass index, total serum cholesterol, blood pressure, and length of education had been obtained at baseline. Blood pressure was considered to be elevated if it exceeded 160 mmHg systolic and/or 95 mmHg diastolic. Presence of macular degeneration in at least one eye of a participant was assessed concurrently from the fundus color photographs by the same examiner.

### Statistical analyses

Distribution of baseline variables was tested using chi squared analysis in the comparison of percentage deviances and variance analysis in the case of continuous variables. Presence of retinal vascular changes was tested for interaction between AT and BC supplementation using the Chi-squared test for two by two tables. Cases were tabulated according to their supplementation with alpha-tocopherol or beta-carotene. When no interaction was observed, the effects of both supplementations were tested by the binomial test. The next step of analysis was carried out using logistic regression analysis with the following explanatory variables: alpha-tocopherol and beta-carotene supplementations, baseline alcohol consumption, cigarette smoking, history of diabetes, claudication and coronary heart disease, total serum cholesterol, body mass index, hypertension, serum and dietary alpha-tocopherol (vit-

amin E) and beta-carotene levels, education, and end-of-trial age and macular degeneration. In most analyses the classes were dichotomized as normal – abnormal, with 0 as normal and 1 and 2 as abnormal.

## Results

Men in the placebo group were slightly younger than those in the other supplementation groups: median age ranged from 67.4 to 68.5 years across the groups.

Baseline serum BC levels was highest among men in the BC group and lowest in the AT group. Other background variables were evenly distributed between the supplementation groups at baseline (Table 1).

Retinal vascular changes were observed in 630 (59%) participants. In 96 % of these cases the changes were present in both eyes. In one subject grade 2 changes were present in one eye, while the other eye showed no changes. The prevalence rate was 40% in the youngest age group (56 to 60 years) rising linearly to 70% in

the oldest age group (76-80 years, Table 2).

Baseline serum and dietary alpha-tocopherol (vitamin E) and beta-carotene were lower among men who later developed retinal vascular changes than in those who did not (Table 3). There was a dose-response relationship, with lower baseline levels associated with more severe retinal vascular changes. Mean visual acuity, as measured by a study nurse 1-12 months before the ophthalmological examination, was 1.137 in eyes with no retinal vascular changes, 1.099 in grade I eyes, and 1.028 in grade II eyes (measured with any glasses used for far vision, average of right and left eyes).

The prevalences of end-of-trial retinal vascular changes were higher in the AT and BC supplementation groups than in the AT plus BC and placebo groups, although not statistically significantly (Table 4). There was no evidence of interaction between the two supplements in their effect on retinal vascular changes ( $p=0.08$ ).

The prevalence rate of end-of-trial retinal vascular changes was 58% among men who received alpha-tocopherol compared to 59% among men who did not (OR 0.96, 95% CI 0.75-1.22). Among men who received BC the prevalence was 58%, compared to 59% among men who did not (OR 0.96, 95% CI 0.75-1.22).

No association of either AT or BC supplementation with the prevalence of retinal vascular changes was observed in the logistic regression analysis adjusting for the background characteristics (Table 5). We calculated the OR's separately for normal (class 0) versus any abnormality (class 1 or 2), and for normal plus class 1 versus class 2. The results were similar, and those for the former comparison are presented in Table 5.

**Table 1.** Baseline characteristics of the participants in the ophthalmological study by supplementation with alpha-tocopherol (AT), both AT and BC, beta-carotene (BC), or placebo (medians or proportions).

	AT n=262	ATBC n=291	BC n=265	Placebo n=254	P-value
Alcohol use (g/day)	12.4	8.3	8.9	10.9	0.31
Elevated blood pressure (%) <sup>1</sup>	31.3	22.7	29.4	23.6	0.06
Serum cholesterol (mmol/L)	6.2	6.2	6.3	6.2	0.91
History of diabetes (%)	6.1	3.8	4.2	3.2	0.38
Cigarettes smoked/day	15.0	15.0	15.0	15.0	0.76
Body Mass Index (kg/m <sup>2</sup> )	25.1	25.3	25.3	25.2	0.99
Education (total years)	8.0	8.0	8.0	8.0	0.49
History of claudication (%)	6.5	6.5	4.2	5.9	0.61
History of coronary heart disease (%)	12.2	11.7	9.8	11.4	0.84
Serum alpha-tocopherol (mg/l)	12.1	11.9	12.1	11.7	0.83
Serum beta-carotene (µg/l)	167.0	184.0	208.0	192.5	0.04
Dietary vitamin E (mg/day)	10.9	10.8	11.0	10.8	0.85
Dietary beta-carotene (mg/day)	1.8	1.9	2.0	2.0	0.28
Age (years) <sup>2</sup>	68.5	68.1	68.2	67.4	0.05

<sup>1</sup> Systolic >160 mmHg and/or diastolic >95 mmHg

<sup>2</sup> At the time of ophthalmological examination

**Table 2.** Retinal vascular changes by age.

Age	Vascular changes		
	No	Rate (%)	Total
56-60	20	40	49
61-65	38	48	80
66-70	346	58	602
71-75	188	66	287
76-80	38	70	54
Total	630	59	1072

**Table 3.** Baseline serum level and dietary intake of alpha-tocopherol (vitamin E) and beta-carotene by the severity of end-of-trial retinal vascular changes.

Retinal vascular changes	Serum AT(mg/l)	Dietary		
		BC(µg/l)	AT(mg/day) (vitamin E)	BC(mg/day)
grade 0	12.4	194.0	11.1	1.92
grade I	11.8	178.0	10.7	1.88
grade II	11.3	173.0	10.8	1.85

**Table 4.** Retinal vascular changes after an average of 6.6 years supplementation, by intervention group (AT=alpha-tocopherol, BC=beta carotene, ATBC=combination of alpha-tocopherol and beta carotene, and placebo).

Intervention group	Retinal vascular changes		
	No	Rate (%)	Total
AT	161	62	262
ATBC	161	55	291
BC	163	62	265
Placebo	145	57	254

**Table 5.** Association of alpha-tocopherol or beta-carotene supplementation with the prevalence of moderate to severe retinal vascular changes in logistic regression analysis adjusting for background variables. Data on background variables are from the baseline, except age and macular degeneration which are from the end-of-trial ophthalmological examination.

Variable	OR	CI 95%
Alpha-tocopherol supplemented vs not supplemented	0.90	0.68–1.19
Beta carotene supplemented vs not supplemented	1.00	0.75–1.33
Age (years)		
–70	1.00	
71–75	1.49	1.08–2.06
76–80	2.07	1.04–4.12
History of diabetes (yes/no)	0.65	0.31–1.38
History of claudication (yes/no)	0.90	0.51–1.59
History of coronary heart disease (yes/no)	0.90	0.57–1.40
Hypertension <sup>1</sup> (yes/no)	1.33	0.96–1.85
Macular degeneration (yes/no)	1.49	1.09–2.05
Cigarettes smoked/day		
–14	1.00	
15–24	0.94	0.66–1.35
25–	0.83	0.52–1.32
Use of alcohol (gm/day)		
Abstainers	1.00	
0–4.6	0.88	0.57–1.37
4.7–19.4	1.07	0.60–1.90
19.5–	1.07	0.38–3.03
Serum cholesterol (mmol/L)		
–5.7	1.00	
5.8–6.7	1.25	0.78–1.99
6.8–	1.87	1.07–3.28
Body mass index (kg/m <sup>2</sup> )		
–23.8	1.00	
23.9–26.9	1.23	0.85–1.79
27.0–	1.22	0.88–1.70
Education (years)		
–8	1.00	
9–11	1.10	0.76–1.59
12–	1.85	1.17–2.93
Serum alpha-tocopherol (mg/l)		
–10.8	1.00	
10.9–13.3	0.83	0.57–1.21
13.4–	0.63	0.39–0.99
Serum beta-carotene (µg/l)		
–139	1.00	
140–252	0.55	0.38–0.79
253–	0.68	0.46–1.00
Dietary vitamin E (mg/day)		
–8.9	1.00	
9.0–13.5	1.03	0.72–1.46
13.6–	0.98	0.67–1.44
Dietary beta-carotene (mg/day)		
–1.4	1.00	
1.5–2.5	0.87	0.62–1.23
2.6–	0.92	0.63–1.34

<sup>1</sup>Systolic blood pressure >160mmHg and/or diastolic >95 mmHg

## Discussion

Supplementation with 50 mg/day of alpha-tocopherol or 20 mg/day of beta-carotene was not associated with end-of-trial prevalence of retinal vascular changes among smoking men. We are not aware of a similar study against which to

compare our results, but while evidence of a beneficial effect of antioxidants in the prevention of atherosclerosis is accumulating from both animal and observational human studies, every effort should be taken to utilize controlled clinical trials to test the efficacy of antioxidants in disease prevention.

AT and BC are naturally occurring antioxidants. Both the serum levels and dietary intakes of AT (vitamin E) and BC at baseline were lower among participants who later developed retinal vascular changes, compared to those who did not. High baseline serum levels of both substances were also associated with a lower risk of retinal vascular changes after adjustment in the logistic regression model for several known risk factors. While the intervention with these agents did not affect the risk of developing retinal vascular changes, one possible explanation is that other dietary or lifestyle factors which are associated with serum and dietary AT (vitamin E) and BC have the protective effect on retinal vascular changes. Previous studies have shown a reduced risk of cardiovascular events with higher plasma levels of both AT (Riemersma et al. 1991) and BC (Riemersma et al. 1991, Street et al. 1991). Food frequency analyses of alpha-tocopherol (Manson et al. 1991) and beta-carotene (Gaziano et al. 1992) intakes have indicated similar protection with higher intake levels.

The prevalence of retinal vascular changes in this study was higher than in similar population-based studies in the United States (Klein et al. 1994) and Sweden (Svardsudd et al. 1978). This may be partly because our participants were older men who had smoked for an average of 36 years.

The observed associations of elevated blood pressure, age and serum cholesterol with retinal vascular changes were as expected. These changes have been widely used to classify the severity of hypertension (Svardsudd et al. 1978). The most severe form of retinal vascular changes, retinopathy (defined as microaneurysms, blot hemorrhages, cotton wool spots, exudates, intraretinal microvascular abnormalities, venous beading and neovascularization of the retina), has been shown to correlate most strikingly with arterial pressure (Klein et al. 1994). Arteriolar narrowing has been shown to be a less prominent sign of hypertension, and arteriovenous nicking the least prominent. Retinopathy, arteriolar narrowing and arteriovenous nicking were more prominent in subjects whose hypertension was refractory to antihypertensive medication (Klein et al. 1994). These changes also occur in persons not suffering from hypertension. They are believed to be a consequence of other processes, such as decreased internal carotid artery

perfusion, aging, and atherosclerosis (Hollenhorst 1958, Kearns 1963, Dimmitt et al. 1989).

The present study is an end-of-trial examination of a sample of participants in a controlled clinical trial of supplementation with AT or BC for an average of 6.6 years. The drop-outs from the ATBC study before the last follow-up visit may have caused a bias in our results, although they were evenly distributed across the four supplementation groups. We studied those in the Helsinki and Uusimaa area who had dropped out of the trial at any time during follow-up; we found that the drop-outs were less compliant, consumed more alcohol, had higher body mass index, smoked more, and had a higher diastolic blood pressure than those who remained in the ATBC study for the whole follow-up period. Interestingly, the drop-outs had lower serum cholesterol than those who continued in the study. There was no difference in systolic blood pressure between the two groups. The risk factors were measured at the start of supplementation. Changes in risk factors during the follow-up were not addressed in our study. However, there is no plausible reason to suggest a supplementation-specific change in risk factors during the follow-up. Moreover, retinal vascular changes were rather prevalent in our study, more than half of all participants showing signs of them. We controlled for major risk factors for retinal vascular changes in the logistic regression analysis. Many of these factors, such as macular degeneration and histories of claudication and coronary heart disease are themselves different expressions of degenerative processes. To avoid "overcontrolling", we tested the data also with basic models having only a few risk factors and found the same result.

Potential bias is eliminated partly by the double-blind design. Study participants and personnel were masked for the supplementation of each participant. Yellowing of the skin was reported in 34% of the ATBC study participants receiving beta-carotene and in 7% of participants not taking beta-carotene. Persistent yellowing of the skin (during two-thirds or more of the follow-up visits) was reported in 8.8% of the participants receiving beta-carotene, and in 0.3% of those who did not. The person classifying the fundus photographs, did not see the study participant at any time, however. The eye fundus of the men showed no sign of coloration.

Adherence to the treatment was excellent in the study. Four out of five active

participants took more than 95 per cent of their scheduled capsules, as calculated from the residual-capsule counts. No difference in compliance was observed between treatment groups (ATBC study group 1994b). Serum concentrations of AT and BC increased substantially in the groups receiving these agents, while they remained stable in the groups not receiving them.

There are no data available on the rate of development of retinal vascular changes over time. All participants of the ophthalmological study had been taking the study supplements for at least 5 years. The duration of intervention may have been too short to affect the course of retinal vascular changes in hypertensive and atherosclerotic processes.

Our conclusion is that long-term supplementation with either alpha-tocopherol or beta-carotene does not affect the occurrence of retinal vascular changes among smoking males.

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